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(54) Title: GLYCOPROTEIN AND/OR GLYCOMACROPEPTIDE CONTAINING OPHTHALMIC PREPARATION

(57) Abstract: The present invention relates to an aqueous fonnulation to be instilled into the eye, or in which to pre soak or store an object to be inserted into the eye, such as a contact lens, an ointment, or a solid device to be inserted into the conjunctival sac. The preparations disclosed are utilized for the treatment of a tear film and ocular surface disorder known as keratoconjunctivitis sicca or dry eye syndrome. In general, the preparations of this invention are also effective for the relief of symptoms of eye irritation, such as those caused by dry environmental conditions or by contact lens wear. In accordance with the present invention, the ophthalmic preparation includes a mucin component, similar to that found at the nonnal human ocular surface and in one exemplary and preferred embodiment, the glycoprotein and/or glycomacropeptide is derived from mammalian milk, preferably bovine.

stability and function of the entire tear film. Recent studies of the tear film using laser interferometry and confocal microscopy might be including the entire gel layer in indicating that the human tear film is 30 to 40 microns thick, more than four times thicker than earlier estimates.

Based on tear film physiology and clinical observations, tear film abnormalities are commonly designated by focus on a specific deficiency, such as an aqueous tear deficiency, kerato-conjunctivitis sicca (KCS), a mucin deficiency, a lipid abnormality, an impaired lid function, or an epitheliopathy. Although clinically useful, the simplistic concept of a lack of one component of the tear film as the cause of dry eye has given way to a much more sophisticated view of ocular surface disease that involves: (1) the health and regulation of the various glands contributing secretions to the tear film, (2) changes in the tear film itself, such as in osmolality and content of inflammatory mediators, and (3) what is viewed as a sort of "final common pathway", the subsequent changes to the ocular surface. In fact, many clinicians and authors prefer the term "ocular surface disease" over "dry eye", for it is change to the ocular surface, whatever the original cause, that results in the significant signs and symptoms of dry eye. The discomfort of ocular surface disease is expressed in ocular symptoms, such as dryness, grittiness, burning, soreness or scratchiness, with variation among individuals. These symptoms can also be exacerbated by factors such as environmental conditions and contact lens wear. The combination of varying clinical signs and symptoms has also been termed dry eye syndrome.

Over the past twenty to thirty years many attempts have been made to provide an effective and long lasting treatment of dry eye symptoms, particularly for patients with moderate to severe KCS. These prior art attempts can be categorized on the basis of their physical state: ointments, emulsions, solid devices and aqueous based solutions or gels.

Ointments are generally anhydrous preparations based on mixtures of white petrolatum and mineral oil. Because these formulations are greasy and cause blurred vision, they are not widely used other than in cases of severe symptoms, and are mostly limited to application at night just before sleeping. Emulsion based formulations for treating dry eye symptoms have emerged over the past ten years. One approach has been disclosed in a series of U.S. patent Nos.: 5,578,586; 5,371,108; 5,294,607;

5,278,151; 4,914,088, all of which are herein incorporated by reference in their entirety. These patents teach the methods and compositions for reducing evaporation of the aqueous layer from the surface of the eye. The method comprises applying an admixture of a charged phospholipid and a non-polar oil over the eye, preferably in the form of a finely divided oil-in-water emulsion. Another approach is described in U.S. patent Nos. 4,818,537 and 4,804,539, incorporated herein by reference in their entirety, where liposome compositions in the form of emulsions are claimed to provide enhanced retention on ocular surfaces and thereby alleviate the symptoms of dry eye.

Solid devices, in the form of ocular inserts, have been utilized for longer term symptomatic relief of dry eye. These devices are placed in the eye and slowly dissolve or erode to provide a thickened tear film. Often patients find these devices difficult to insert and once in place, they tend to be uncomfortable.

The most recommended and commercially successful methodology to treat dry eye symptoms is aqueous based solutions or gels. For the patient, eye drops are convenient and easy to apply relative to the other options mentioned above. There are at least thirty artificial tear products currently on the market from which to choose. For the most part the "active" ingredients in these present day artificial tear formulations are common water soluble or dispersible polymers such as: hydroxyethylcellulose; hydroxypropylmethylcellulose; methylcellulose; carboxymethylcellulose; polyvinyl alcohol; polyvinyl pyrrolidone; polyethylene glycol; carbomers; and poloxamers.

These currently marketed products, while providing temporary relief of symptoms - usually measured in minutes - are strictly palliative without long term effect. In fact, to truly maintain relief of symptoms in moderate to severe cases, an impractical schedule of doses would be necessary. With preserved solutions, the frequency of instillation can lead to signs and symptoms of irritation, making it necessary to utilize expensive and more cumbersome unit dose delivery packages.

The recent patent literature indicates a continued interest in pursuing synthetic based artificial tear solutions. For example, U.S. patent No. 5,460,834, incorporated herein by reference in its entirety, teaches the use of hydroxypropylmethylcellulose along with other ingredients as an ophthalmic solution, and U.S. patent No. 6,180,093 incorporated herein by reference in its entirety, discloses the use of polyvinylpyrrolidone in combination with other components to relieve eye dryness.

The art recognizes that an ophthalmic solution must provide an effective and long lasting treatment for symptoms of dry eye. One approach to achieving these aims is to provide a solution with tailored rheological properties, that is, a high viscosity solution that yields or flows under stress. Examples of this approach are disclosed in U.S. patent Nos. 5,075,104 and 5,209,927, incorporated herein by reference in their entirety, where the rheological properties of the ophthalmic solutions are attained through the use of carbomer polymers. These carbomer polymers have been found to be bio-adhesive as described in U.S. patent Nos. 5,225,196, 4,983,392 and 4,615,697, all of which are incorporated by reference in their entirety. It is believed that the bio-adhesive properties of the carbomer contributes to longer retention times in the eye. In fact, U.S. patent Nos. 5,075,104 and 5,209,927, incorporated by reference in their entirety, teach "that the carbomer polymers appear to function by maintaining or restoring the normal hydration equilibrium of the epithelial cells, thus protecting the cornea.

The search for useful ophthalmic solution polymers has extended into the area of bio polymers, with particular emphasis on the naturally occurring polysaccharides. One polymer, hyaluronic acid, and its sodium salt have received much attention over the past several years. In fact, one commercial product, Hylashield®, based on a high molecular weight sodium hyaluronate, has been successfully marketed as a dry eye treatment solution. The use of hyaluronic acid in artificial tear solution compositions is also taught in U.S. patent 5,460,834 which is incorporated by reference in their entirety. Other polysaccharides, such as carrageenan, tamarind gum and keratan sulfate have been claimed to have utility in artificial tear solutions as disclosed in U.S. patent Nos. 5,403,841 and 5,460,834, and 6,056,950, all of which are incorporated by reference in their entirety. In addition, polysaccharides, such as alginate, dextran, scleroglucan and xanthan have been used, or have been proposed for use in ophthalmic solutions.

The patent literature reveals one dated reference to the use of mucin in sterilized, preserved and stable solutions. U.S. patent No. 4,438,100, incorporated herein by reference in its entirety, describes mucin-containing solutions for application to sensitive mucous membranes of the oral cavity, the nasal system and the eye. The mucins utilized in this invention are non human mammalian mucins selected from the group consisting of buccal and gastrointestinal mucins. In fact, the source of their

mucins is mucus, a mature and complex secretion containing a mixture of various mucin molecules as well as other proteins and associated contaminants of secretion. There is no distinction made between secreted mucins and mucins expressed by the surface cells of the oral cavity or gastrointestinal mucous membranes. One very recent application, U.S. patent No. 6,281,192, incorporated by reference in its entirety discloses ophthalmic applications of mucin derived from mammalian milk or milk byproducts: the mucin described was found to be a MUC1 type mucin similar to the transmembrane mucin expressed on the surface of the human eye.

SUMMARY OF INVENTION:

The present application relates to ophthalmic preparations for use as a tear film supplement. More specifically, the invention relates to an aqueous formulation to be instilled into the eye, or in which to pre soak or store an object to be inserted into the eye, such as a contact lens, an ointment, or a solid device to be inserted into the conjunctival sac. The preparations disclosed are utilized for the treatment of disorders such as keratoconjunctivitis sicca or dry eye syndrome. In general, the preparations of this invention are also effective for the relief of symptoms of eye irritation, such as those caused by dry environmental conditions or by contact lens wear.

In particular, the present application relates to ophthalmic compositions including at least one glycoprotein and a glycomacropeptide component, as well as to methods for their preparation and storage. The application also relates to a method of treating the eye by topically applying the composition of the present invention, when indicated, to provide lubrication and protection of the ocular surface, for the relief of dryness and discomfort symptoms, such as experienced in patients with dry eye and following traumatic injury or surgery, and when indicated to achieve the other effects mentioned above. In one preferred embodiment the compositions of the present invention are provided as buffered, sterile aqueous solutions. The subject compositions may be unpreserved (provided in a unit dose format) or may be preserved.

In one preferred embodiment, the glycoproteins and glycomacropeptides are isolated from mammalian milk or milk byproducts. In another preferred embodiment the glycoproteins and glycomacropeptides are isolated from bovine milk. In yet another preferred embodiment the glycoproteins and glycomacropeptides are derived from dairy

whey, a byproduct of cheese making. To form the ophthalmic preparations disclosed herein, other ingredients commonly employed in ophthalmic formulations are utilized to provide a balance of physiologically acceptable properties, depending on whether the final product is a solution, ointment, gel or solid. However, it will be understood that the glycoproteins and glycomacropeptides can be derived from a number of other sources so long as the materials are suitable for the intended use described herein.

The above-discussed and other features and advantages of the present invention will be appreciated and understood by those skilled in the art from the following detailed description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS:

Glycroproteins are of great interest as components of mammalian membranes. Structurally they are composed of relatively short carbohydrate sequences covalently linked to a protein core. The glycoproteins of the cell surface appear to function in recognition, intercellular adhesion and modulation of certain intracellular transport event. That one characteristic phenotypic expression of tumorigenic transformation is an alteration in cell surface glycoproteins strongly implies an involvement of glycoproteins in at least several cell surface functions. It is believed that the glycoproteins and glycomacropeptides of this invention leave biological activity and many stimulate the production tears and in particular the stimulation of mucus.

Lipid globules in milk are enclosed in a membrane which is derived directly from the apical plasma membrane of mammary epithelial cells. This milk fat globule membrane (MFGM) can be readily obtained in quantity, and MFGM from bovine milk exhibits a higher degree of purity with respect to apical surface origin of the membrane material. Thus MFGM is an excellent source material for isolation of glycoproteins associated with the cell surface or, more properly, with a derivative of the apical cell surface. Over the past twenty years, there have been numerous publications elucidating both the isolation procedures for and the characterization of glycoproteins from milk.

With bovine MFGM, the presence of five to eight major glycoproteins have been detected with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of these glycoproteins can range from about 30,000 to 40,000 daltons up to about 250,000 daltons. The more abundant glycoproteins have

molecular weights in the 60,000 to 125,000 dalton range. A mucoprotein has also been isolated from bovine milk and was found to have a molecular weight of about 123,000 daltons.

The glycoproteins isolated form bovine milk and milk byproducts can have carbohydrate contents, as a percentage of the protein weight, of 10% to 50% or even higher. The water solubility or compatibility of the glycoproteins increases with increasing carbohydrate content. In fact, Kobylka et al back in 1972 reported the isolation and characterization of six major glycoproteins from the bovine milk fat globule membrane. The molecular weights of these are listed below.

Glycoprotein	Molecular Weight (daltons)
I	240,000
II	155,000
III	92,000
IV	80,000
V	65,000
VI	53,000

When isolated from milk fat globules, the glycoproteins may exist as a complex. This complex may contain mucin associated with one or more other glycoproteins and other components such as lipids, phospholipids, lipoproteins and oligosaccharides. These complexes can have an apparent molecular weight of from about 200,000 to 500,000 daltons or greater. In the practice of this invention either the glycoproteins themselves or the complex of glycoproteins can be utilized.

Bovine milk and milk byproducts also contain naturally occurring glycomacropeptides. The most notable is a sixty-four residue glycopeptide with a relatively high molecular weight. Glycomacropeptides are also produced by cleaving the peptide linkages of kappa casein. The cleavage process can be carried out either chemically through hydrolysis or enzymatically utilizing chymosin. The resulting glycomacropeptides structurally are composed of carbohydrate moities covalently linked to relatively short peptide sequences. The molecular of theses

glycomacropeptides is generally in the 3,000 to 15,000 daltons and more typically in the 6,000 to 10,000 dalton range.

Mucins refer to a family of glycoproteins of high molecular weight, secreted or expressed by goblet and nongoblet epithelial cells of mucosal tissues. These mucins predominate in the formation of mucus, a highly hydrated gel of particular structure and function. Mucins are heavily glycosylated high molecular weight glycoproteins with predominantly O-linked oligosaccharide side chains. Mucin molecules are generally above 200Kda, with carbohydrate composing 50% or more of their dry weight. At least nine distinct mucin genes have been identified (MUC1, 2, 3, 4, MUC5AC, MUC5B, MUC6, 7 and 8). Furthermore, each of these is produced in various forms in different tissues. This suggests that the mucins serve unique, tissue-specific protective functions at the apical surfaces of specialized epithelial cells. MUC1, the so-called "epithelial mucin", is a membrane-spanning mucin found in abundance in epithelial tissues. MUC1 is known by various other names, such as polymorphic epithelial mucin or episialin. MUC1 has a molecular weight in the 125 to 225KDa range, which is low when compared to the other types of mucin.

In the human eye, the secretory mucins MUC2 and MUC5AC have been detected (via transcripts at the nucleic acid level) from conjunctival isolates, and only MUC5AC has been localized to conjunctival goblet cells. The transmembrane mucin MUC1 is associated with the cell membranes of the entire corneal and conjunctival epithelial surface, except the goblet cells. The transmembrane mucin MUC4 is associated with the cell membranes of the entire conjunctival epithelial surface, except the goblet cells. Qualitative and quantitative analyses of ocular mucins are difficult because there are as yet no specific probes for individual ocular mucins, and little is known about the mechanisms or extent of synthesis or secretory regulation of these mucins. One or more calcium dependent processes are possible, with involvement of several secretagogues, such as prostanoids, autonomic transmitters, and neuropeptides being investigated.

The secreted ocular mucins are relatively large molecules, and have a significant role in the gel-forming nature of the tear film. The model of the greater part of the tear film being a highly hydrated mucus gel, rather than simply a watery aqueous layer, is becoming increasingly accepted. The viscoelasticity of the tear film derives from the

specific structure and gel-forming properties of the ocular mucins, and allows the tear film to absorb the shear force of the blink, which would otherwise irritate and damage the ocular surface. The transmembrane mucin, on the other hand, serves more as a protective layer on the actual cellular surface of the ocular epithelium, functioning to directly protect and lubricate the ocular surface, as well as to anchor the highly hydrated gel (mucus) of the tear film gel-forming mucins, thereby assisting in the spreading and stability of the tear film over the ocular surface..

Stratified layers of the above mucins are known to form over the surface of mucosal membranes, such as in the gut, affecting the flow and interaction of the protective layer and its contents with the cellular surface of the epithelium. A difficiency in one type of mucin would therefore be expected to affect the lubricating, protective, barrier and other functions of the other mucins at the mucosal surface.

In a mild to moderate dry eye, the goblet cell density is not significantly reduced, indicating that MUC5AC is most likely still able to be produced normally, in quantities sufficient to be spread over the entire ocular surface. However, localized early ocular surface changes resulting from dryness, such as that revealed by fluorescein or rose bengal staining, can be seen in the epithelia of the corneal and conjunctival surfaces. This localized damage to the ocular surface indicates that even marginal dryness might have a significant effect on the presence of functional MUC1 on the surface of the ocular epithelium. Since one of the proposed functions of MUC1 is to help the other, more abundant gel-forming ocular mucins adhere to the ocular surface, a paucity of MUC1 might significantly affect the stability of the tear film, even in the presence of an abundance of MUC5AC secreted by the conjunctival goblet cells. When investigated using the technique of impression cytology, the more severe ocular surface changes resulting from dryness, exhibited in the process of squamous metaplasia, are also seen to occur initially in localized areas. These more pathological localized surface changes are further evidence for a critical protective role of MUC1. There is some early evidence that with the progression of changes to the ocular surface mucins associated with dry eye, as detected by immunohistochemical methods, the goblet cells themselves try to make up for the lack of normal expression of MUC1 by the rest (non goblet cells) of the corneal and conjunctival surface epithelium, and begin expressing a MUC1-like molecule in their secretions.

Although not being held to any one theory we believe that the glycoproteins and glycomacropeptides described in this invention, being components of mammalian membranes, act to protect and lubricate the ocular surface, as in the role of the natural transmembrane mucins, MUC1 and MUC4, which is expressed by the entire surface epithelium of the conjunctiva and cornea. By supplementing the natural epithelial surface mucin, the lubrication and protection of the ocular surface is enhanced, in order to slow the progression, and associated development of symptoms, of changes to the ocular surface epithelium, such as decreased tear film stability, increased staining with fluorescein sodium or rose bengal, decreased goblet cell density and the development of squamous metaplasia seen with ocular surface disease. The property of viscosity in the preferred embodiment is primarily targeted to assist in retention of the invention in the eye at the ocular surface, as well as for lubrication and comfort associated with instillation. Viscosity is not the physical property which gives the mucin formulation of this invention its "mucomimetic" function. This invention primarily protects and lubricates the ocular surface and interacts with the gel-forming secreted mucins of the tear film, thereby enhancing the spreading of the tear film, and by default of instillation adds to the tear film volume and hydration of the ocular surface. The "mucomimetic" effects of this invention protect the ocular surface from dryness and absorb shear forces of the blink, and assist the eye's own secreted gel forming mucins (predominantly MUC5) in maintaining their viscoelastic properties and ensuing structure and stability of the tear film, thereby slowing or preventing the changes to the ocular surface seen in dry eye conditions.

The glycoproteins and glycomacropeptides of this invention are preferentially isolated from bovine milk or milk byproducts. Most preferably the glycoproteins and glycomacropeptides are isolated from dairy whey.

Vista Scientific has applied for and has received allowance for the trademark "Milcin". Milcin™ refers to the glycoproteins and glycomacropeptides isolated from milk or milk byproducts. For the purpose of this invention the term "Milcin™" will be used to describe glycoproteins and glycomacropeptides isolated from dairy whey. The material Milcin™ is utilized as the active ingredient in ophthalmic preparations.

Recovery and purification of glycoproteins and glycomacropeptides can be carried out utilizing standard methods known in the art. These would include, but are

not limited to, membrane filtration and microfiltration, tangential flow filtration, chromatography (e.g., size exclusion, ion exchange, affinity), extraction, adsorption, precipitation (with non-solvents, salts, etc.), density gradient fractionation, electrophoresis, electrodialysis, isoelectric focusing, acid or base hydrolysis, and chymosin hydrolysis.

The scientific literature reveals a number of techniques for characterizing the various types of glycoproteins and glycomacropeptides. These techniques include, but are not limited to, chromatographic techniques or gel electrophoresis, particularly SDS-PAGE, followed by direct protein staining (e.g. silver staining) or immunohistochemical staining (e.g. Western blotting or Northern blotting), immunoprecipitation techniques, amino acid analysis, carbohydrate determination, lectin binding probes, light scattering, scanning electron microscopy, mass spectrometry, protein nitrogen content and ash.

The amount of glycoprotein and/or glycomacropeptide in an ophthalmic formulation can vary greatly depending on the product type. For example, in contact lens related solutions the glycoprotein and/or glycomacropeptide concentration would vary from about 0.0001% to 5.0% by weight. In dry eye preparations the glycoprotein and/or glycomacropeptide level could vary from about 0.1% to about 10.0% by weight. In a solid ocular insert delivery device the glycoprotein and/or glycomacropeptide level could range to about 90% or greater by weight. Within each type of preparation the concentration might be varied, depending on such factors as the severity of the dry eye condition being treated, to enhance particular properties of the glycoprotein and/or glycomacropeptide solution. These ranges are for the purpose of illustration and are not meant in any manner to limit the scope of the claims.

Exemplary ophthalmic compositions include a glycoprotein and/or glycomacropeptide from any number of the exemplary sources described herein before. In addition, other solution components may be employed as required:

VISCOSIFIERS

Cellulose derivatives are commonly used to increase viscosity. Specific cellulose derivatives include: hydroxypropylmethylcellulose, carboxymethylcellulose, methylcellulose, hydroxyethylcellulose, etc. Some polysaccharides may also be utilized

to increase the viscosity of ophthalmic solutions and include xanthan, scleroglucan, carrageenans, tragacanth gum, hyaluronic acid etc. Other viscosifiers that are useful include polyvinylpyrrolidone, polyvinyl alcohol, polyethyleneoxide, polyacrylic acid and crosslinked polyacrylic acid. Generally, viscosifiers are present in the amount of 0.1 to 0.75 % by weight of the solution.

BUFFERING AGENTS

Any pharmaceutically acceptable buffer system may be utilized and include phosphates, borates, citrates, acetates and carbonates in amounts necessary to produce a pH of about 6.0 to about 8.0.

TONICITY AGENTS

The tonicity of the ophthalmic solutions described here can be adjusted to either hypotonic, isotonic or hypertonic relative to normal tears by use of generally used materials know to the art. Sodium and potassium chloride are widely used to adjust tonicity. Other agents include dextrose, mannitol, sorbitol and urea.

HUMECTANTS

Water binding compounds aid in retaining moisture on the ocular surface and include glycerin, propylene glycol, polyethylene glycol.

WETTING AGENTS

Certain compounds are useful to promote surface wetting, whether it be the ocular surface or the surface of a contact lens. One category that is preferred is the polyoxamers. These polyethyleneoxide-polypropyleneoxide-polyethyleneoxide block copolymer are available from BASF. Other compounds include the Tetronics®, reverse Pluronics® and the reverse Tetronics®, also available from BASF.

PRESERVATIVES

The compositions of this invention may include a preservative in an effective amount. Preservatives known to the art include alkyldimethyl benzylammonium chloride (BAK), chlorhexidene gluconate (CHG), polyhexamethylene biguanide

(PHMB), other polyquats and sorbic acid. The subject compositions may also include a co-preservative and/or chelating agent, such as ethylenediaminetetraacetic acid (EDTA) and its salts.

OTHER ADDITIVES

In some cases it may be beneficial to include other components in an ophthalmic solution. These include specific ions, such as Ca++, Zn++ and Mg++, Cu++, selenium, vitamins, such A, C and E, to promote ocular health. The compositions described in this invention may also be utilized as vehicles for drug delivery. Drugs often used in the eye include anti-glaucoma compounds, anti-inflammatory agents and anti-infective agents.

As previously described, this invention finds particular utility as lubricating eye drops, i.e., an artificial tear solution, a tear fluid supplement, a delivery vehicle for topical ophthalmic drug application. In most of these applications, the compositions of this invention are provided in a buffered, sterile aqueous solution. Typically, these solutions have a viscosity from about 1 to 100 cps. As a solution the compositions of this invention are dispensed in the eye in the form of an eye drop. It should be understood, however, that the compositions described in this invention may also be formulated as viscous liquids, i.e., viscosities from several hundred to several thousand cps, gels or ointments. In these applications the mucin component would be dispersed or dissolved in an appropriate vehicle such as Lubragel, GRR Lubricating Jelly or Karajel, all trademarked products of United-Guardian, Inc., Hauppauge, NY.

The compositions of this invention may also be formulated as solid ocular inserts that dissolve or erode over time when placed in the cul-de-sac of the eye.

Swelling-controlled release devices would consist of mucin homogeneously dispersed in a glassy polymer such as a water soluble cellulosic. When the insert is placed in the eye, the tear fluid begins to penetrate the matrix, followed by swelling, and finally dissolution, of the matrix. As this process occurs, mucin is released into the eye to provide relief of dry eye symptoms over a long period of time.

Erodible devices would again consist of glycoprotein and/or glycomacropeptide homogeneously dispersed in a polymer matrix. In this case, glycoprotein and/or glycomacropeptide is released by a chemical reaction (hydrolysis) that results in

solubilization of the matrix polymer, usually at the surface of the device. Generally, the matrix material is a polyanhydride, poly(ortho ester), polylactic acid or a polyglycolic.

In another embodiment the glycoprotein and/or glycomacropeptide may be chemically modified or crosslinked to act as its own "matrix", where the glycoprotein and/or glycomacropeptide comprises the entire, or nearly entire, device, thus providing the maximum amount of glycoprotein and/or glycomacropeptide available to the eye.

Furthermore, in some contact lens related embodiments, the glycoprotein and/or glycomacropeptide disclosed herein may be incorporated into contact lens soaking and conditioning solutions as well as lubricating eye drops for contact lens wearers.

In another embodiment the glycoproteins and/or glycomacropeptides may be utilized in drug delivery. The most common and convenient method for delivery of ocular drugs is by way of topical eye drops. Generally, the solution vehicles employed are quickly diluted by the tear fluid and drain from the eye in a matter of minutes. This short residence time hinders the absorption and hence the bioavailability of the drug in the eye. Oftentimes the short residence time is overcome by greatly increasing the concentration of the drug to improve bioavailability. This often leads to significant undesirable side effects due to the systemic actions of many of the ocular drugs currently prescribed.

Much research has been done to improve the residence time of the drug vehicle at the ocular surface and also to promote interaction or association of the drug with the vehicle. One approach that has been commercialized is to utilize a crosslinked carboxy-functional polymer such as Carbopol®, supplied by B.F. Goodrich. The bioadhesive nature of this polymer has been the basis for controlled release ophthalmic formulations as described in U.S. 4,615,697 and U.S. 5,188,826, both of which are incorporated by reference in their entirety. These crosslinked carboxy-functional polymers swell in aqueous solution but remain as micron-size hydrated particles. Furthermore, at neutral pH, they are substantially anionic in nature. Since many ophthalmic drugs, for example timolol and pilocarpine, are positively charged, they will associate with the negatively charged polymer particles through electrostatic interaction. Also, since the hydrated particles are microporous, the drug can be absorbed into the matrix. When an ophthalmic solution of this type is placed in the eye, the hydrated polymer particles adhere to the mucosal surface, providing extended

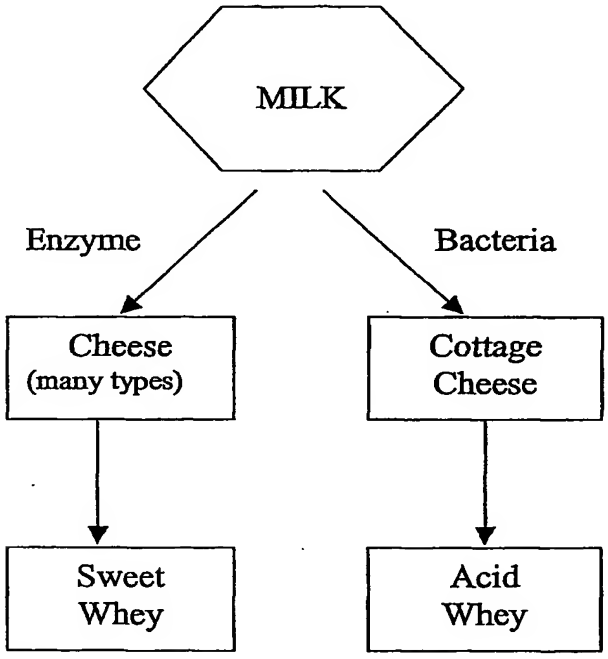
residency time. During this residence the drug is released from the hydrated polymer particles, thus providing for a more efficient local delivery to the eye.

The glycoproteins and/or glycomacropeptides of the present invention are considered "bioadhesive" given their functions in association with the plasma membrane of mammary epithelial cells. Given this information one would expect the glycoproteins and/or glycomacropeptides of this invention to act in a similar manner to the crosslinked carboxy-functional polymers as an ophthalmic drug delivery vehicle. In practice, the glycoproteins and/or glycomacropeptides of this invention provide superior retention time due to their ability to interact not only with the epithelial surface but also with the natural mucins in the tear film.

The following examples are merely illustrative of glycoprotein and glycomacropeptide containing ophthalmic preparations and the examples should not be considered as limiting the scope of this invention in any way.

EXAMPLE 1

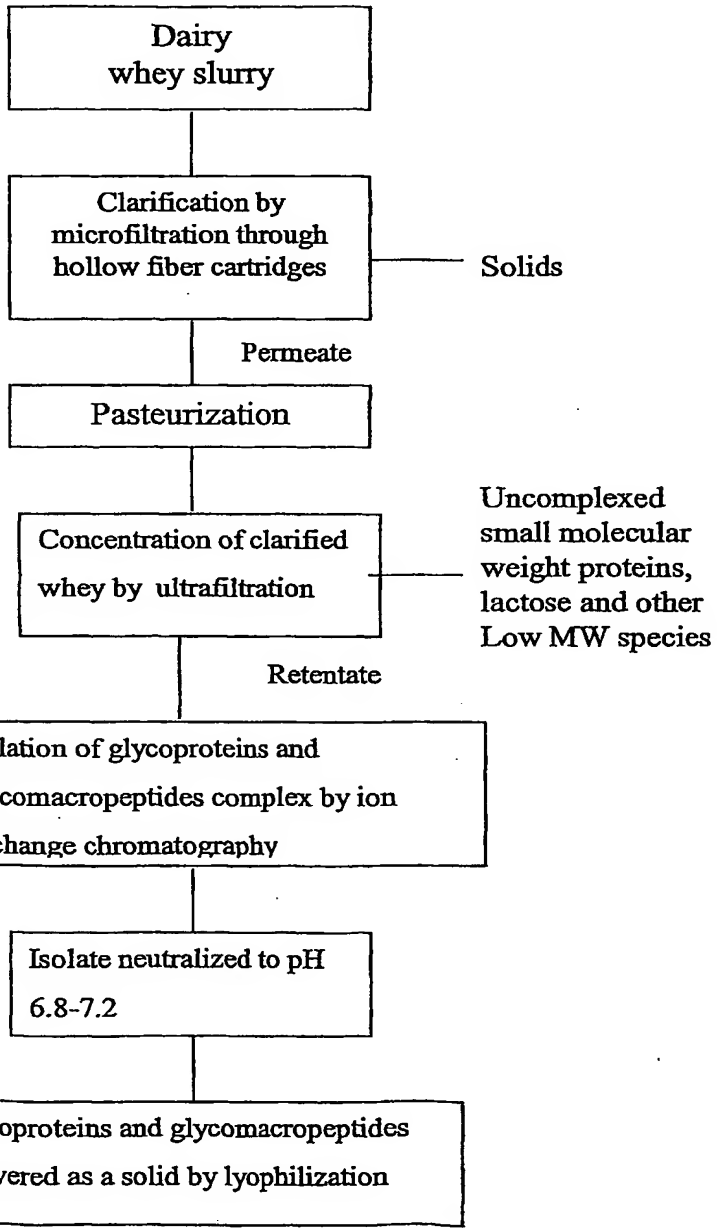
Whey is the by product of cheese manufacturing which begins with milk and can be divided into two processes, where the major one involves enzyme treatment of milk and the minor one involves bacteria treatment.



Sweet whey accounts for about 80% of whey produced and is an inexpensive and readily available source of glycoproteins and glycomacropeptides.

EXAMPLE 2

This example outlines a process for recovering glycoproteins and glycomacropeptides from dairy whey. For this example sweet dairy whey is the starting material, although acid whey can also be utilized since they both contain the glycoproteins and glycomacropeptides of this invention.



EXAMPLE 3

The following table describes the physical and chemical properties of the glycoproteins and glycomacropeptides derived from dairy whey.

Composition	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Protein (%)	67.0	60.7	61.4	67.4	66.4	66.3
Lipid (%)	21.0	22.2	25.6	19.4	22.1	19.7
Ash (%)	3.5	4.3	3.5	3.7	3.8	3.6
Moisture (%)	5.9	7.8	1.9	2.2	2.2	4.1
pH	—	6.7	6.9	6.9	6.9	6.9

Notes:

Above in percent by weight.

Protein determined by Leco combustion.

Lipid determined by Mojonnier method.

Moisture determined by weight loss under heat and vacuum.

Ash determined by AOAC ignition test.

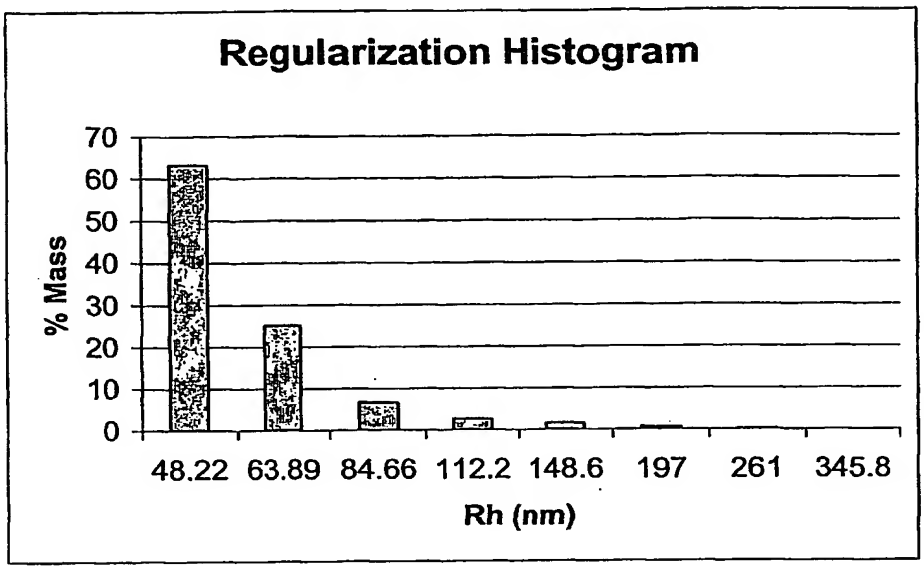
pH determined on 1% concentration in phosphate buffer solution.

The above described glycoproteins and glycomacropeptides are referred to as "Milcin™", a trademark of Vista Scientific.

EXAMPLE 4

The glycoproteins and glycomacropeptides described in Example 3 are complexes composed essentially of the protein with physically associated lipids. This complex exists in aqueous solution as a hydrocolloid. A 1% by weight of Milcin™ Lot 4 (Example 3) dispersion was prepared in borate buffer, pH 7.2 and osmolality of 300. Particle size of the Milcin™ hydrocolloid was then determined by dynamic light scattering utilizing a Protein Solutions DynaPro MS/X instrument equipped with a Peltier temperature control device. The resulting data was analyzed with the Dynamics

version 5.26.38 software provided by the manufacturer. Results were obtained using the regularization method at a grid size of 100. The following histogram was generated by the data.

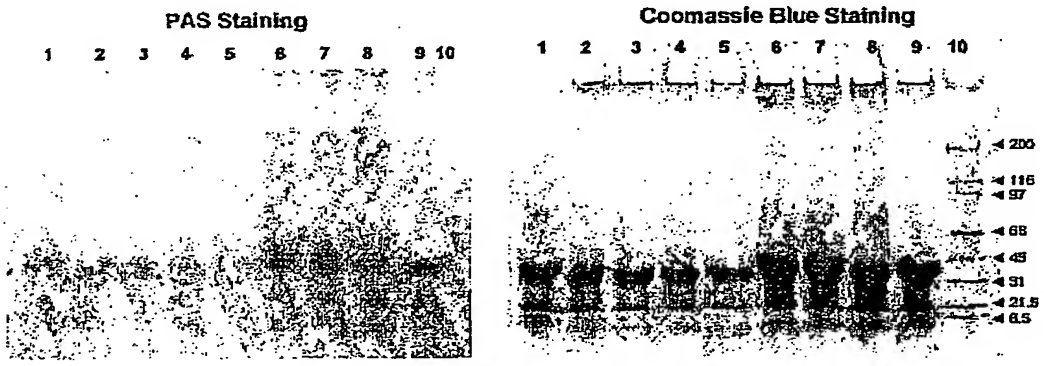


From the results it can be seen that the bulk of the Milcin™ hydrocolloid has a hydrodynamic radius of about 40nm to about 60nm. There is a small amount of material that has a hydrodynamic radius about 100nm.

EXAMPLE 5

SDS-PAGE was utilized to separate the glycoproteins and glycomacropeptides contained in the Milcin™ complex as a function of molecular weight. This was accomplished by rendering a negative charge in the proteins through their interactions with sodium dodecylsulfate (SDS). When placed in the gel and an electric field applied, the proteins migrate as a function of their charge to molecular weight. By providing all the proteins with the same charge with SDS the proteins migrate through the gel as a function of molecular weight only, and became distributed in the bands according to molecular weight. Known amounts of protein molecular weight standards were run concurrently to demonstrate molecular weight position. The resulting gels

were first oxidized then stained with a Schiff reagent to develop only the glycoprotein bands (stains glycoproteins pink). After removing the pink stain, the gel is restained with coomassie blue stain. The coomassie blue reagent reveals only the protein bands (stans proteins blue). In this manner both the glycoprotein and other proteins that are present in the Milcin™ material are identified. The following diagram presents the molecular analysis of a number of Milcin™ samples (see Example 3). It can be seen that the lots of Milcin™, are for the most part, composed of glycosylated protein. The molecular weight of these glycosylated proteins range from about 2000 daltons (glycomacropeptides) to about 200,000 daltons (mucin). The bulk of the Milcin™ composition appears to be glycoprotein in the molecular weight range of about 20,000 to 100,000 daltons.



EXAMPLE 6

The following example illustrates a method for extracting the lipids from Milcin™. A sample of Milcin™ Lot 4 (see example 3) was rigorously extracted in the following manner. Approximately 1.0 gm of Milcin™ (Example 3 Lot 4) was extracted with methanol for one hour and the solids filtered. The solids were then resuspended in 15ml of chloroform and extracted for one hour and the solids filtered. The solids were then resuspended in 15ml of hexanes and extracted for one hour. The solids were filtered and dried in vacuum for one hour. The resulting sample was lipid free.

EXAMPLE 7

Glycoproteins and glycomacropeptides are proteins, or protein fragments, that contain carbohydrate side groups. The amount of carbohydrate can vary widely from 10% or less to over 50% by weight of the total glycoprotein or glycomacropeptide. Glycoproteins and glycomacropeptides typically contain N-linked and O-linked oligosaccharides. This example presents the analysis of the extracted Milcin™ of Example 6 for both neutral monosaccharides and sialic acid content. A sample of the lipid extracted Milcin™ of Example 6 was submitted to:

Glyco Solutions Corp
25 Winthrop St.
Worcester, MA

for analyses. The results are presented below.

Neutral Monosaccharide Analysis (pmol/8 μg sample)

Fucose	BLQ* (80pmol)
GAlNac	775 pmol
GlcNAc	573 pmol
Galactose	1183 pmol
Mannose	696 pmol

* Below the limit of qualification

From the above values it is calculated that there was 0.636 μg carbohydrate/8 μg sample, or 8% neutral carbohydrate. This calculation for total carbohydrate excludes any charged monosaccharides (sialic acids, for example) that are not measured by this analysis.

Sialic Acid Analysis (pmol/8 μg** sample)

NeuAc	1100 pmol
NeuGc	16.5 pmol

** Values were normalized to 8 μg of sample to make it consistent with previous monosaccharide composition values.

The NeuAc value was measured from the injection of 12.5% of the hydrolysate and the NeuGc value was measured from the injection of 25% of the hydrolysate. From the above values it was calculated that there was 0.346 μg sialic acid/8 μg sample, or 4.3% sialic acid.

From the above data the sample (lipid free Milcin™) contains approximately 12 to 13% by weight total carbohydrate. The results depend on the assumption that all of the carbohydrate has been hydrolyzed. If this is not the case then the true carbohydrate content will be higher.

EXAMPLE 8

Milk products generally contain lactose unless it is specifically removed. This example presents data on the content of lactose in the Milcin™ glycoprotein and glycomacropeptide complex. A sample of the lipid extracted Milcin™ of Example 6 was analyzed for lactose content by:

Glyco Solutions Corp
25 Winthrop St.
Worcester, MA

The analysis revealed that the Milcin™ sample contained no lactose (less than 34 ug of lactose per 100 mg of sample). The following indicates that the process for recovering Milcin™ glycoprotein and glycomacropeptide complex from dairy whey effectively excludes lactose as an impurity.

EXAMPLE 9

The glycoproteins and glycomacropeptides of this invention as isolated from dairy whey contain a small amount of lipids that are complexed with the protein. The following example illustrates the extraction of lipids from Milcin™ and the subsequent identification of those extracted lipids by liquid chromatography (TLC).

Silica gel plates were activated at 100°C for 30 minutes and kept in a vacuum dessicator. Chromatograms were developed using various hexanes/diethyl ether solvent systems and visualized by staining with a saturated ethanolic phosphmolybdic acid solution. The lipid fraction was probed for each class of lipids by comparing the R_f values with control lipids in an appropriately chosen solvent system. The table below summarizes the results of the extractions and chromatograms. Each lot (see Example 3) was successively extracted three times with methanol. For the mass determination, all the methanol fractions were combined, solvent removed *in vacuo* and the residue was weighed.

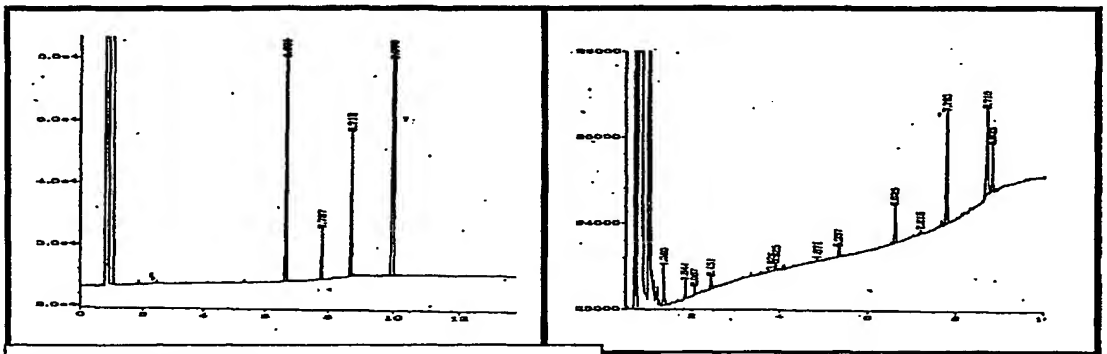
	Milcin™ Lot 1	**Milcin™ Lot 2**
Sample Weight	995mg	1020mg
Glycoprotein	781mg	800mg
Lipids	108mg	173mg
TLC Results	MeOH: alkyl esters, triglycerides, fatty acids and polar esters.	MeOH: alkyl esters, triglycerides, fatty acids and polar esters

As the results indicate Milcin™ contains about 10 to 20% by weight of loosely bound lipids, that is, those extractable by methanol. These lipids are primarily alkyl esters, fatty acids, polar esters and triglycerides.

EXAMPLE 10

The lipids extracted from Milcin™ in Example 6 were subjected to analysis by gas chromatography. A series of lipids, Tripalmitate, Phosphotidylcholine dimyristate, Cholesterol oleate and Arachidic acid in a ratio of 1:10:3:30, [based on fatty acid

content], were subjected to transmethylation. The resulting fatty acid methyl esters were run on High Resolution Gas Chromatography and yielded the FAME standards shown below. The retention time for each of the peaks on the H-P 1 column is proportional to the molecular weight of the FAME. Integration of these peaks showed a quantitative recovery of the starting lipids as their fatty acid methyl esters. A similar extract and transesterification of the lipids recovered in Example 6 gave the FAME profile shown below. In the extracted lipids from Example 6, we can identify myristate, palmitate and oleate. No arachidate was found.



FAME Standards of
Methyl myristate [C_{14}] Ret Time = 6.60min
Methyl palmitate [C_{16}] Ret Time = 7.78 min
Methyl oleate [C_{18}] Ret time = 8.71 min
Methyl arachidate [C_{20}] Ret time = 9.97 min

Extracted Fames from
Milcin™ Lot 4
(see example 6)

EXAMPLE 11

The glycoproteins and glycomacropeptides of this invention would be expected to contain common metal ions. To verify the presence of these metal ions, direct current plasma (DCP) emission spectroscopy was utilized. The technique is essentially an atomic absorption technique where the spectral emission is formed in a plasma between the anode and cathode of a coupled electrode. The technique is quantitative. Milcin™ Lot 4 (see Example 3) was dissolved in de-ionized water at a concentration of 1,000ppm. Nitric acid was added (1ppm). Proper DCP procedure was followed, including a two-point calibration (10ppm, 0ppm) for multi-element analyses. The results are tabulated below and reflect the averaging of six measurements for each metal.

Metal	Measured Concentration* (ppm)	Percent by Mass in Pure Milcin™
Magnesium	0.67 ± 0.02	0.067% (670ppm)
Potassium	1.8 ± 0.1	0.18% (1,800ppm)
Sodium	8.6 ± 0.3	0.86% (8,600ppm)
Copper	0.06 ± 0.20	0.006% (60ppm)
Zinc	0.2 ± 0.2	—

* the measured concentration is the concentration of the metal in a 1,000ppm solution of pure Milcin™ Lot 4.

EXAMPLE 12

The following example illustrates the use of the glycoproteins and glycomacropeptides of the invention as the active ingredients in an ophthalmic solution for the treatment of dry eye signs and symptoms. The formulation listed below:

INGREDIENTS	AMOUNT IN GRAMS
Milcin™ Lot 4	1.05
Hydroxyethylcellulose Natrosol 250M Pharm	0.4
Sodium chloride	0.48
Sodium borate decahydrate	0.12
Boric acid	0.74
Water	97.21

was prepared in the following manner:

1. Weigh out the water and add to an appropriate beaker equipped with a stirring apparatus.

2. With stirring, add the sodium chloride, sodium borate decahydrate and boric acid to the water.

3. Stir until the salts have completely dissolved, about 10 minutes.

4. With vigorous stirring, add the Milcin™ slowly to the batch.

5. Continue vigorous stirring until the Milcin™ is finely dispersed, about 30 minutes.

6. With moderate stirring, add the hydroxyethylcellulose slowly to the batch.

7. Continue stirring at a moderate rate for 2 hours.

8. Allow the batch to deaerate for 15 minutes.

9. Place batch in a suitable sealed container for autoclaving.

10. Autoclave the batch for 60 minutes at 121°C.

11. Remove solution container from the autoclave.

12. Open container and perform batch testing.

Once prepared, the formulation listed above was tested and the following solution properties were determined.

PROPERTY	VALUE
Viscosity, cps	24
Osmolality, mOsm/kg	304
pH	7.2
Appearance	Slightly hazy
Color	Bluish tint

EXAMPLE 13

This example illustrates the ocular compatibility of the glycoproteins and glycomacropeptides of this invention utilizing an *in vitro* transepithelial permeability assay.

The irritation potential of Milcin™ was evaluated at a level of 1.05% in an ophthalmic solution. The commercially available lubricant eye drop solution "Refresh Tears", manufactured and sold by Allergan, was also evaluated.

The following solution formulations were prepared utilizing the compounding procedure detailed in Example 12. The finished solutions were transferred aseptically into sterilized bottles.

INGREDIENT	A	B
Milcin™ Lot 4,gms	—	1.05
Hydroxyethylcellulose Natrosol 250M Pharm, gms	0.40	0.40
Sodium chloride, gms	0.48	0.48
Sodium borate decahydrate, gms	0.12	0.12
Boric acid, gms	0.74	0.74
Water, gms	98.26	97.21

The solutions described in the table above were subjected to the following experiments to determine potential eye irritation of the solutions. The experimental methods follow the procedure developed by R. Tchao, which is described in "Trans-Epithelial Permeability of Fluorescein *In Vitro* as an Assay to Determine Eye Irritants", Progress in *In Vitro* Toxicology, Volume 6, 1988, pages 271-283 (Mary Ann Liebert, Inc. Publishers, New York), the disclosure of which is incorporated herein by reference.

The Tchao technique is described as a method of determining potential eye irritation of a substance by correlating damage to a mono-layer of Madin-Darby Canine Kidney (MDCK) cells with damage to corneal epithelial cells. The amount of fluorescein passing through the cell mono-layer is a function of permeability of the cell mono-layer. Higher cell mono-layer permeability indicates greater damage to the cell junctions from application of a test solution thereto, whereas lower cell mono-layer permeability indicates less damage to the cell junctions from application of the test solution.

The details of the test are presented below.

Culture preparation: MDCK cells are obtained from ATCC, and maintained in minimum essential medium (MEM) supplemented with 10% bovine calf serum with iron supplementation (Hyclone, Utah). Stock cultures are passaged weekly using trypsin and EDTA. Cultures are used before passage 50. For the test, 0.5ml of a cell suspension containing 2 x 10 E5 cells are seeded in Millicell HA 13mm inserts (Millipore, Bedford, MA). The inserts are placed in 24-well plates and fed with 0.5ml medium. Two days after seeding the cells, the media both inside and outside the inserts are replaced with fresh media. On day 6 after seeding, the inserts are used for testing the solutions. It has been shown that the resistance developed by a confluent MDCK monolayer is about 600 ohms/cm^2.

Test: Each insert is rinsed with Hanks Balanced Salt Solution (HBSS) 3 x 1 ml using a 10 ml syringe without needle. Each test solution (0.5 ml) is added to the inside of an insert that has been placed in a fresh 24-well plate. Triplicate inserts are used for each test solution. The 24-well plate with inserts and test solutions are placed in a humidified incubator at 37°C for 30 minutes. Each series of triplicates is handled sequentially to allow exact timing of the treatment. After incubation, sequentially, each insert is individually rinsed with HBSS 5 x 1 ml using the 10 ml syringe, and is placed in a fresh 24-well plate containing 0.5 ml HBSS in each well. 0.5 ml of a solution of Na-fluorescein (3 mg/100 ml) is added to each rinsed insert. After incubation at room temperature for 30 minutes, the inserts are sequentially removed from the wells, and the amount of Na-fluorescein in each of the wells is measured in a CytoFluor 2300, using

540 nm excitation and 590 nm emission. For each test, the negative control is HBSS and the positive control is 250 μg/ml sodium dodecyl sulfate (SDS). It has been determined that the assay can measure the effect of 50 μg/ml SDS, and the effect on the permeability of the monolayer is linearly proportional to the concentrations of SDS from 50 – 250 μg/ml. Fluorescence units (arbitrary) of each test solution is plotted against test solutions.

Interpretation of results: The results are expressed as % of SDS response, and comparisons with the HBSS response. Generally, if the solution is 20% of the SDS response, the solution may be a mild irritant.

The results of the *in vitro* irritation potential testing are presented below along with the results for the positive and negative controls. The positive control 250 ppm of sodium dodecyl sulfate (SDS) is known to cause noticeable irritation when instilled in the human eye. The negative control Hank's balanced salt solution (HBSS) is known not to elicit any adverse reaction when instilled in the human eye. The results are expressed as a percentage of SDS response, that is, SDS = 100% response. Any response less than 20% indicates little or no tissue change and is considered non-irritating.

SOLUTION	RESPONSE
SDS (250ppm)	100
Solution A	0.64 ± 0.1
Solution B	0.86 ± 0.1
Refresh Tears	2.45 ± 0.5
HBSS	3.00 ± 0.5

It can be seen that the response of the Milcin™ solution (B) is the same as the control solution (A) and both are well below the response of the negative control HBSS. Given this data, Milcin™ based ophthalmic solutions should be completely compatible with the ocular environment.

EXAMPLE 14

The following example illustrates the robustness of the glycoprotein and the glycomacropeptide of this invention with respect to autoclaving. The formulation listed below:

INGREDIENT	AMOUNT IN GRAMS
Milcin™ Lot 4	1.05
Hydroyethylcellulose Natrosol 250M Pharm	0.40
Sodium chloride	0.48
Sodium borate decahydrate	0.12
Boric acid	0.74
Water	97.21

was prepared by the detailed process given in Example 12 except that half the batch was autoclaved and half the batch was not (steps 1 through 8 only in Example 12).

Once prepared, both formulations representing both process conditions were tested and the following solution property was determined.

	Before Autoclaving	After Autoclaving
PROPERTY	VALUE	VALUE
Viscosity, cps	37	24
Osmolality, mOsm/kg	293	304
pH	7.2	7.2
Appearance	Slightly hazy	Slightly hazy
Color	Bluish tint	Bluish tint

The above results confirms the robustness of Milcin™ and demonstrate the ability of Milcin™ containing ophthalmic solution to be sterilized by autoclaving.

EXAMPLE 15

The following example illustrates the use of lipid free glycoproteins and glycomacropeptides in an ophthalmic solution. The extracted glycoproteins and

glycomacropeptides recovered in Example 6 were utilized as the active ingredients in the ophthalmic solution formulation described below:

INGREDIENT	AMOUNT IN GRAMS
Extracted Milcin™ (see Example 6)	1.00
Hydroxyethylcellulose Natrosol 250M Pharm	0.50
Propylene glycol	0.50
Sodium chloride	0.20
Sodium borate decahydrate	0.12
Boric acid	0.70
Potassium sorbate	0.15
Edetate disodium	0.05
Water	96.78

was prepared by the detailed process given in Example 12. The finished solution was tested and the following physical properties were generated.

PROPERTY	VALUE
Viscosity, cps	27.0
Osmolality, mOsm/kg	317
pH	6.6
Appearance	Clear
Color	Water white

EXAMPLE 16

The example illustrates the preparation of a preserved ophthalmic solution utilizing the glycoproteins and glycomacropeptides of this invention.

INGREDIENT	AMOUNT
Milcin™ Lot 6	3.00gms
Sodium chloride	0.48gms
Sodium borate decahydrate	0.11gms
Boric acid	0.75gms
Polyhexamethylene biguanide	10 ppm
Purified water	95.66 gms

The above formulation as prepared by dissolving the salts in water followed by the addition of the polyhexamethylene biguanide and then the Milcin™. The batch is

then vigorously mixed for one hour. The resulting solution was translucent with a pH of 6.9 and an osmolality of 305mOsm/kg.

EXAMPLE 17

The following example illustrates the use of glycoproteins and glycomacropeptides in an ophthalmic gel for the treatment of dry eye signs and symptoms. Lubragel® MS, available from United-Guardian, Inc. was chosen as the gel base. Lubragel® MS is composed of polyglycerolmethacrylate and propylene glycol preserved with parabens. A 2% Milcin™ (Lot 6) in Lubragel® MS was prepared by thoroughly mixing the Milcin™ into the gel base to form a uniform dispersion. The resulting gel was slightly hazy.

EXAMPLE 18

This example illustrates the use of the glycoproteins and glycomacropeptides of this invention in contact lens solutions. The contact lens solution base utilized in this example was RENU™ MultiPlus, manufactured and sold by Bausch & Lomb. RENU™ is a multi-purpose solution for the storage and care of soft hydrogel contact lens. The following formulation was prepared by adding 0.5% by weight of Milcin™ to RENU™.

INGREDIENT	AMOUNT IN GRAMS
Milcin™ Lot 6	0.25
RENU™ Lot CH7058	49.75

The Milcin™ was thoroughly dispersed in the RENU™ by vigorous stirring for one hour. The resulting solution was slightly hazy with a pH of 7.1 and an osmolality of 292mOsm/kg. It is expected that the addition of Milcin™ to a soft contact lens solution will result in improved lubricity and comfort to the lens wearer.

EXAMPLE 19

The following example illustrates the use of glycoproteins and glycomacropeptides as an erodible ocular insert to provide long lasting treatment of dry eye. Approximately 50mg of Milcin™ Lot 3 (see Example 3) was placed in a micro KBr press. The press was heated to about 80°C and then tightened to compress the Milcin™ under heat and pressure. The KBr press was allowed to cool to room temperature and the sample was removed. The Milcin™ was in the form of a solid disk about 5mm in diameter and about 2mm in thickness. The Milcin™ disk was placed in water and slowly eroded over about two hours.

EXAMPLE 20

This example illustrates the use of the glycoproteins and the glycomacropeptides of this invention as a component in an allergy relief solution. The particular ingredient for allergy relief chosen was olopatadine hydrochloride. Patanol® is a commercially available solution containing 0.1% by weight of olopatadine. The other solution components are sodium chloride, a phosphate buffer system and benzalkonium chloride as a preservative. Patanol® is an isotonic solution with a pH of about 7. A solution was prepared by adding 1.0% by weight of Milcin™ Lot 6 (see Example 3) into Patanol® solution which is manufactured and sold by Alcon Pharmaceuticals. The Milcin™ was compatible in the Patanol® solution and is expected to provide improved lubricity and comfort to the patient.

EXAMPLE 21

This example illustrates the use of the glycoproteins and glycomacropeptides of this invention in an antibacterial ophthalmic solution with activity against a broad spectrum of gram-postive and gram-negative ocular pathogens. The antibacterial agent chosen was ciprofloxacin hydrochloride. Ciloxan® is a commercially available solution containing 0.3% ciprofloxacin. The other solution ingredients are sodium acetate, mannitol, edetate disodium and benzalkonium chloride. Ciloxan® is an isotonic

solution with a pH about 4.5 that is manufactured and sold by Alcon Pharmaceuticals. A solution was prepared by adding 1.0% by weight of Milcin™ Lot 6 (see Example 3) into Ciloxan® solution. The Milcin™ was compatible in the Ciloxan® solution and is expected to provide improved lubricity and comfort to the patient.

EXAMPLE 22

This example illustrates the use of the glycoproteins and glycomacropeptides of this invention in an antibiotic and steroid combination ophthalmic solution. The antibiotic agent chosen was tobramycin and the steroid chosen was dexamethasone. Tobradex® is a commercially available solution containing 0.3% by weight tobramycin and 0.1% by weight of dexamethasone. The other solution ingredients are hydroxethylcellulose, sodium chloride, sodium sulfate, Tyloxapol®, edetate disodium and benzylalkonium chloride as the preservative. Tobradex® is manufactured and sold by Alcon Pharmaceuticals. A solution was prepared by adding 1.0% by weight of Milcin™ Lot 6 (see Example 3) into Tobradex® solution. The Milcin™ is expected to provide improved lubricity and comfort to the patient.

WHAT IS CLAIMED IS:

CLAIM 1. An ophthalmic preparation comprising at least one material selected from the group consisting of a glycoprotein and a glycomacropeptide derived from a natural source.

CLAIM 2. An ophthalmic preparation comprising at least one material selected from the group consisting of a glycoprotein and a glycomacropeptide component derived from one of mammalian milk and a milk byproduct.

CLAIM 3 An ophthalmic preparation in accordance with Claim 1 wherein the glycoprotein and the glycomacropeptide containing components have a molecular weight of from about 3,000 daltons to about 250,000 daltons.

CLAIM 4 An ophthalmic preparation in accordance with Claim 1 wherein the glycoprotein and the glycomacropeptide containing components have a carbohydrate content of about 5% by weight to about 25% by weight.

CLAIM 5. An ophthalmic preparation in accordance with Claim 1 wherein said preparation is in the form of a solution.

CLAIM 6 An ophthalmic preparation in accordance with Claim 1 wherein said preparation is in the form of an ointment.

CLAIM 7. An ophthalmic preparation in accordance with Claim 1 wherein said preparation is in the form of an ocular insert.

CLAIM 8. An ophthalmic preparation in accordance with Claim 1 wherein the at least one material is present in an amount from about 0.001% to about 1.0% by weight.

CLAIM 9. An ophthalmic preparation in accordance with Claim 1 wherein the at least one material is present in an amount from about 1.0% to about 10.0% by weight.

CLAIM 10. An ophthalmic preparation in accordance with Claim 1 wherein the at least one material is present in an amount from about 10% to about 90% by weight.

CLAIM 11. An ophthalmic preparation in accordance with Claim 1 wherein the at least one material is complexed with at least one component selected from the group consisting of a lipid, phospholipid and lipoprotein.

CLAIM 12. An ophthalmic preparation in accordance with Claim 11 wherein the lipid component is present in the amount of 0.01% to about 30% of the complex.

CLAIM 13. An ophthalmic preparation in accordance with Claim 1 wherein the at least one material is autoclavable.

CLAIM 14. An ophthalmic preparation in accordance with Claim 1 further comprising a buffering agent.

CLAIM 15. An ophthalmic preparation in accordance with Claim 1 further comprising a viscosity modifying agent.

CLAIM 16. An ophthalmic preparation in accordance with Claim 1 further comprising a tonicity modifying agent.

CLAIM 17. An ophthalmic preparation in accordance with Claim 1 further comprising a humectant compound.

CLAIM 18. An ophthalmic preparation in accordance with Claim 1 further comprising a therapeutic drug.

CLAIM 19. An ophthalmic preparation in accordance with Claim 1 further comprising a buffering agent and a viscosity modifying agent.

CLAIM 20. An ophthalmic preparation in accordance with Claim 1 wherein said milk byproduct comprises a milk byproduct in the form of whey.

CLAIM 21. An ophthalmic preparation in accordance with Claim 20, wherein said whey is purified to recover said at least one material.

CLAIM 22. An ophthalmic preparation for treating an eye by topically applying the preparation to an ocular surface to provide lubrication and protection to the ocular surface for the relief of dryness and discomfort symptoms, the ophthalmic preparation, comprising:

a buffered, sterile aqueous solution including at least one material selected from the group consisting of a glycoprotein and a glycomacropeptide, wherein said glycoprotein and/or the glycomacropeptide is derived from one of mammalian milk and milk byproducts.

CLAIM 23 An ophthalmic preparation in accordance with Claim 22, wherein said milk byproduct comprises whey, said whey being purified to recover said glycoprotein and/or glycomacropeptide component.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/31657

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 38/16
US CL : 514/8
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/8, 912; 424/95, 104

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
On-Line Medical Dictionary

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST (USPTO), NPL-Medline, General Internet Search (Yahoo)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	FUJIHARA et al., Lactoferrin suppresses loss of corneal epithelial integrity in a rabbit short-term dry eye model. J Ocul Pharmacol Ther. April 1998; 14(2):99-107. Abstract. Use of lactoferrin (glycoprotein isolatable from bovine milk whey, inherently autoclavable), in a concentration of 0.01 to 1% as a suggested opthlamic preparation for corneal epithelial integrity (i.e. dry eye treatment).	1,2,8,9,13,20-23 — 5-7,10,11-12,14-19
X	CHU et al. Isolation and characterization of porcine milk lactoferrin. Am J Vet Res. July 1993; 54(7):1154-9. P. 1154, left column, first paragraph (lactoferrin "molecular mass is estimated to be between 72,000-85,000 daltons).	3
X — Y	HWANGBO et al. Purification and characterization of novel whey glycoprotein WGP-88 which binds to a monoclonal antibody to PAS-4 glycoprotein in the bovine milk fat globule membrane. Biosci Biotechnol Biochem. September 1997; 61(9): 1568-74. P. 1571, left column, 4th paragraph, glycoproteins WGP-88 contained a 17.1% carbohydrate and PAS-4 had 7.2%. P. 1568 discusses phospholipids and glycoproteins as constituents of bovine milk (which could be isolated alone or together).	4 — 11-12

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US PUB. NO.: US 2002/0045582 A1. (MARGOLIN et al.) 18 April 2002 (18.04.02). Entirety. Stabilized formulation for delivery of any protein therapy that could be a solution, ointment, or ocular insert; and which describes the use alone or in combination of: buffering agents, viscosity modifying agent, tonicity modifying agent, humectant compound, therapeutic drug/carrier.	5-7, 14-19
X	US 6,281,192 B1 (LEAHY et al.) 28 August 2001 (28.08.01). Entirety. (Mucin (which contains glycoproteins) containing ophthalmic preparations).	1-23
X, P	US 6,429,194 B1 (LEAHY et al.) 06 August 2002 (06.08.02). Entirety. Mucin containing ophthalmic preparations. Specifically claim 11 recites glycoprotein content (applicant's core structure), claim 8 autoclavable inherent property (applicant's cl. 13), claim 26 leaves the option for carbohydrates and lipids with glycoprotein (applicant's claims 4, 11-12).	1-23